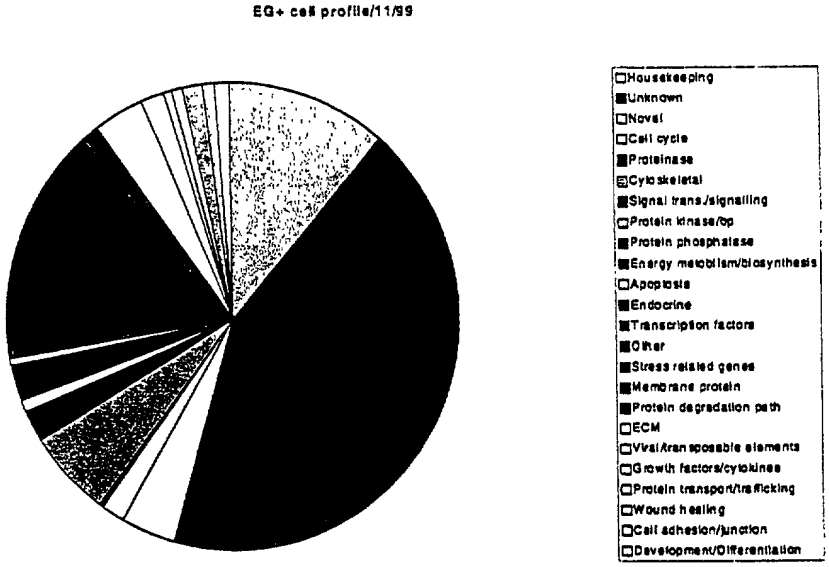
Figure 1: Comparison of EG+ and ES- donor cell expression profiles determined using cDNA microarray, differential display, and direct sequencing methods.



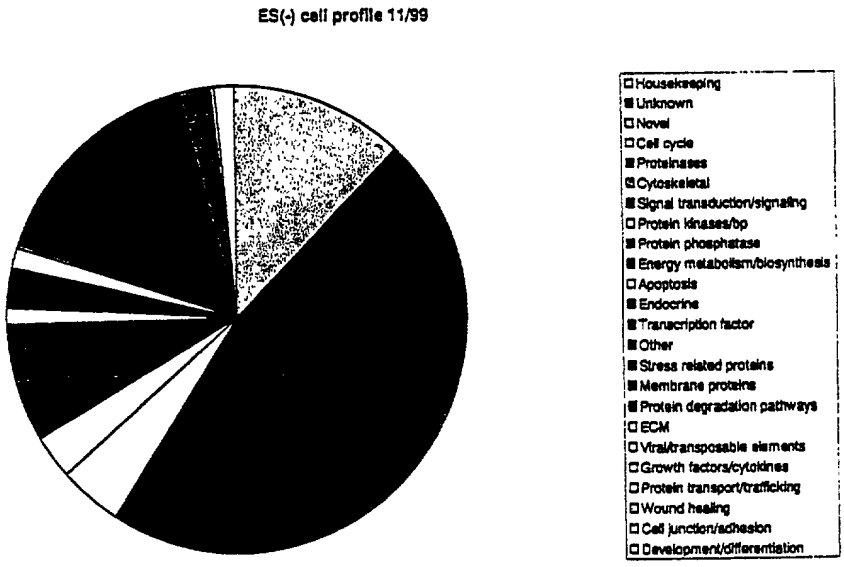


Figure 2: Immunoblot analysis of cultured EG+ and ES- donor cells.

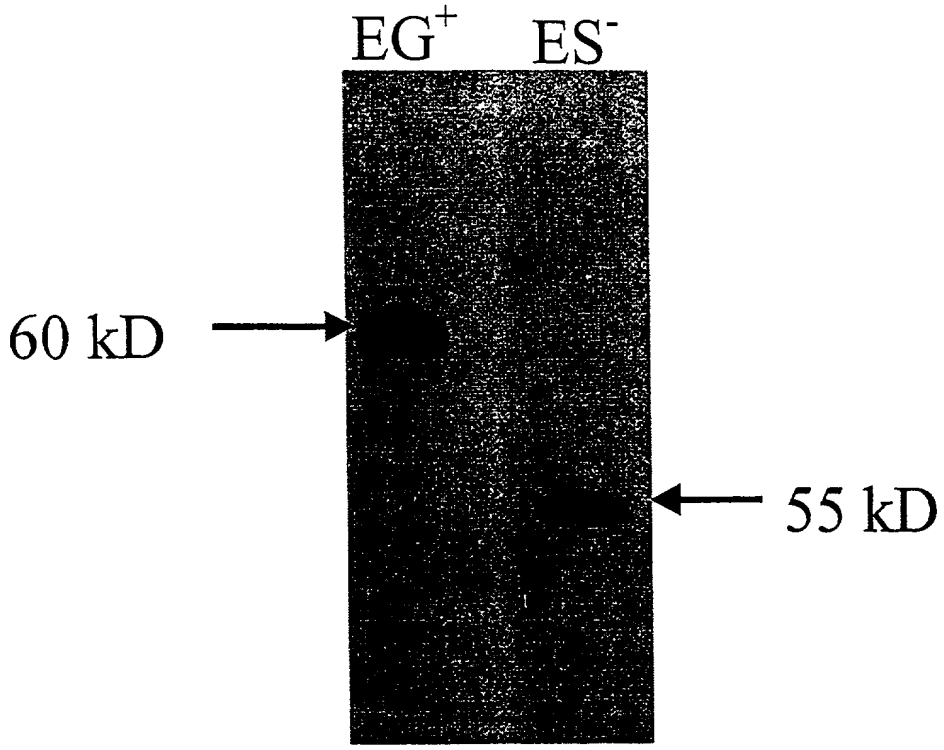


Figure 2: Immunoblot analysis of cultured EG^+ and ES^- donor cells. Samples of cultured donor EG^+ and ES^- cells were subjected to SDS-PAGE and transferred to nitrocellulose. The blot was probed with anti-histone deacetlyase 2 monoclonal antibody. A novel, 55 kD band was detected in ES^- cells but was absent in EG^+ cells. A predicted ~60 kD band was detected in EG^+ cells, but not ES^- cells.

Figure 3: Primer Pairs

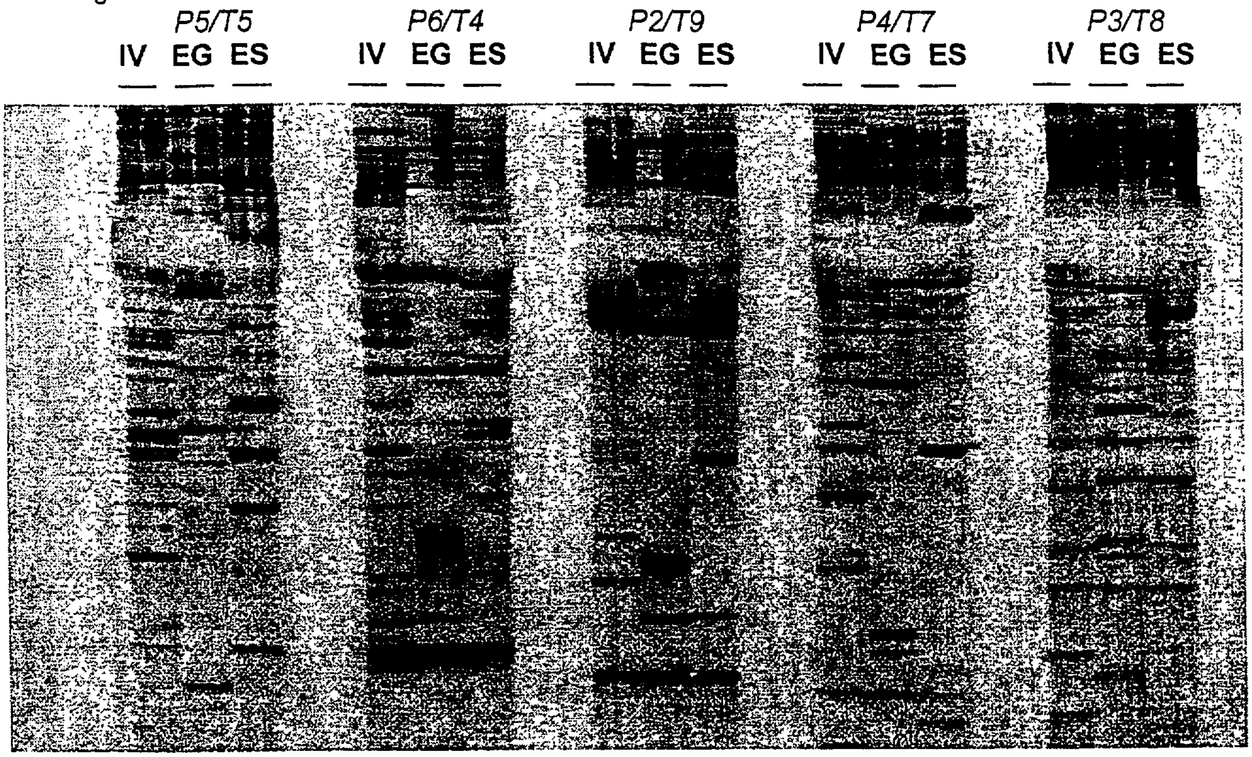


Figure 3. Example of differential display gel comparing mRNA expression patterns of individual in vivo (IV) embryos with individual embryos reconstructed from EG^- and ES^+ donor cell lines using 5 different primer pairs. Boxes represent examples of bands present in individual in vivo embryos, but not detected in individual NT reconstructed embryos.

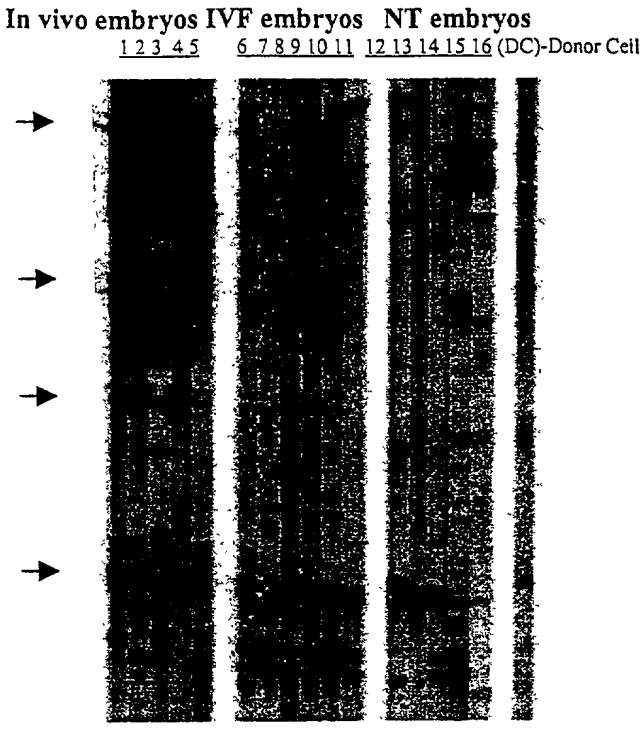


Figure 4A & B. A) Differential Display analysis comparing banding patterns of 5 individual in vivo embryos, 6 individual IVF embryos, 5 individual NT embryos and the donor cell (DC) line used to reconstruct the NT embryos. Arrows indicate bands present in all *in vivo* and at least 5 of 6 IVF produced embryos. B) Histogram indicating the percentage of bands shared with *in vivo* embryos.

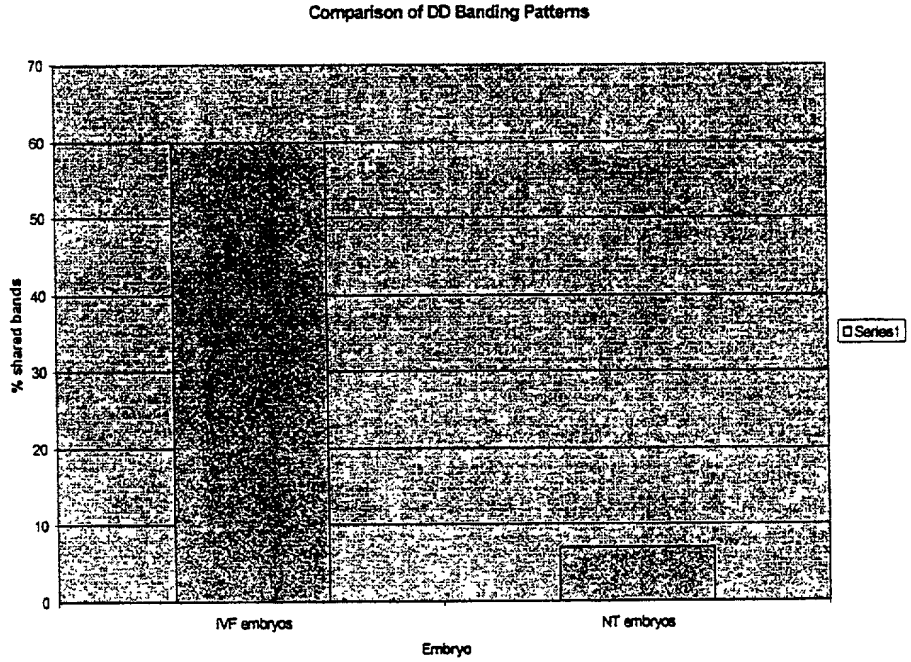
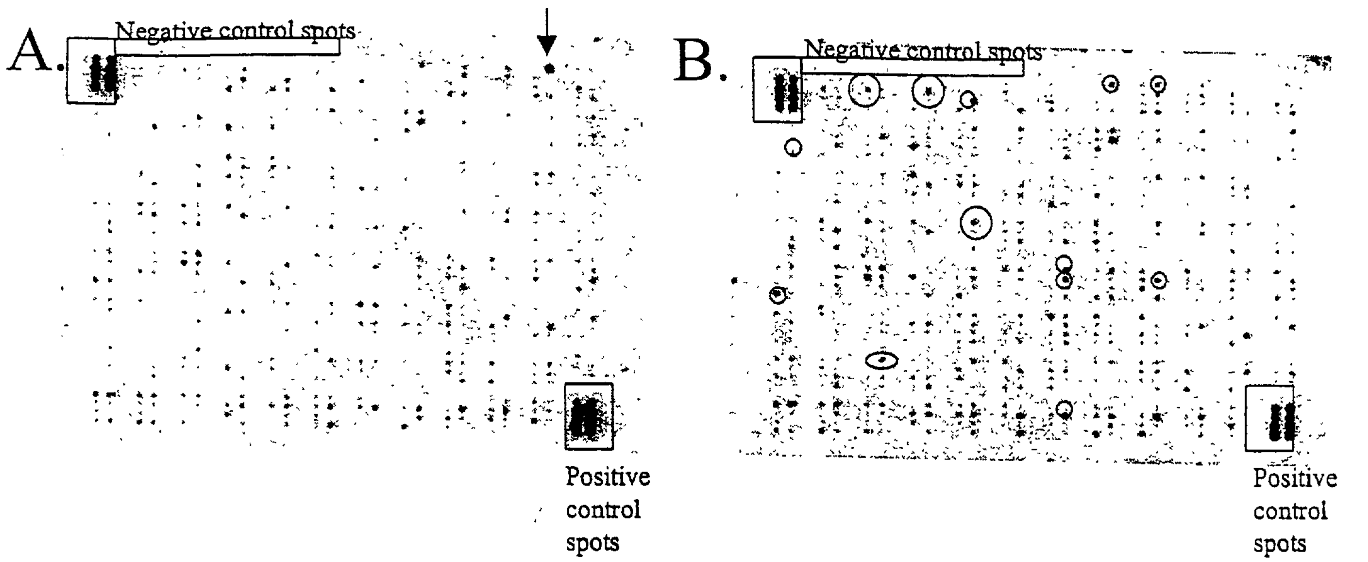
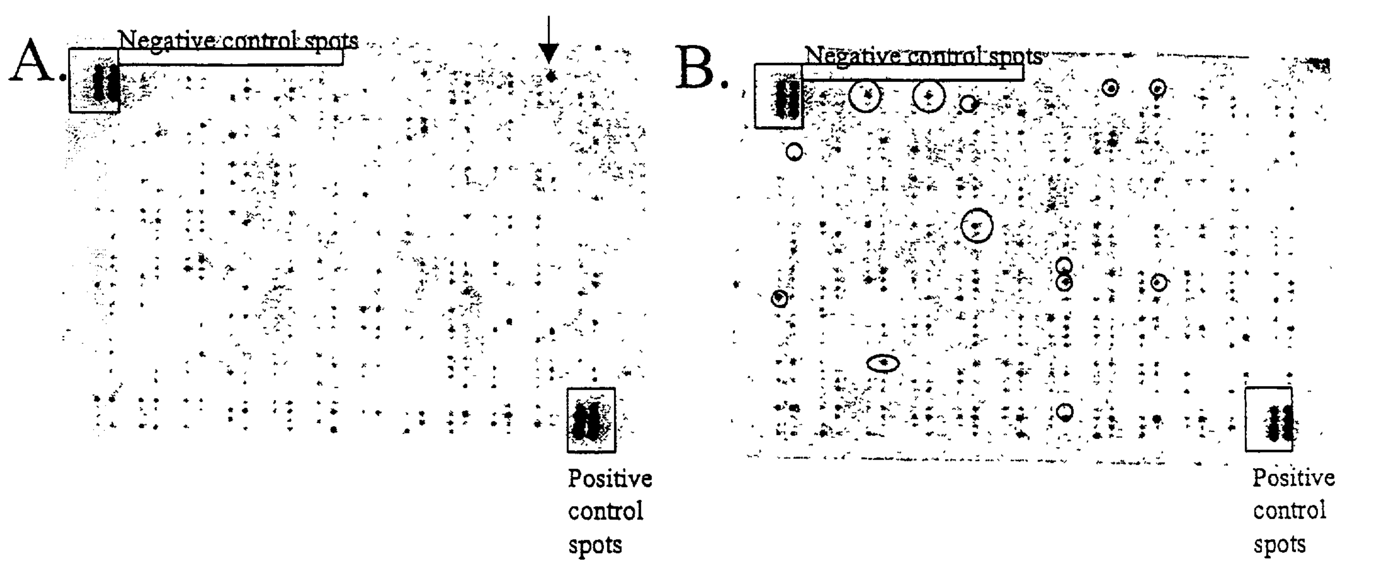


Figure 5. *Examples illustrating identical cDNA arrays probed with mRNA representations of a single NT embryo (A) and a single in vivo embryo (B). Spots enclosed by circles represent clones detected at high levels in a single in vivo embryo and a single NT embryo reconstructed using a competent donor cell line, but at low levels (or undetected) in single NT embryos reconstructed from incompetent donor cell lines and an unknown cell line*



(A single spot displaying the opposite pattern is indicated by an arrow.) Positive control spots are boxed at the corners of the membrane; negative control spots are indicated by rectangles along the top row. The cDNA array was comprised of cDNA clones representing numerous functional classes and gene families, including unknown ESTs, genes putatively associated with reprogramming (SNF2), cell cycle progression (quiescen, cyclins), cell adhesion-extracellular matrix (collagen, fibronectin) apoptosis (p53) imprinting (Igf2 and Igf2r) and transcription (STAT,), embryonic signaling (interferon tau) and signal transduction (JAK).



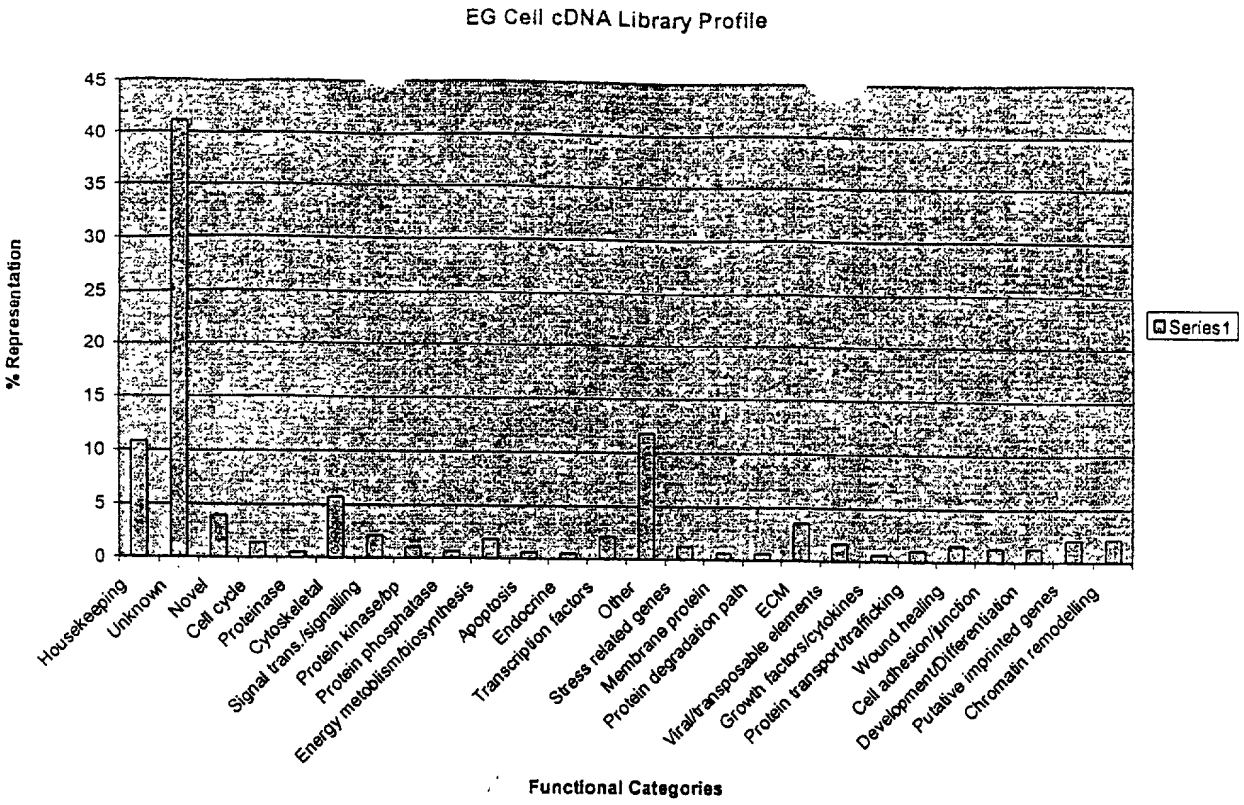


Figure 6: illustrates a profile of the cDNA clones used for micro- and macroarray analysis.

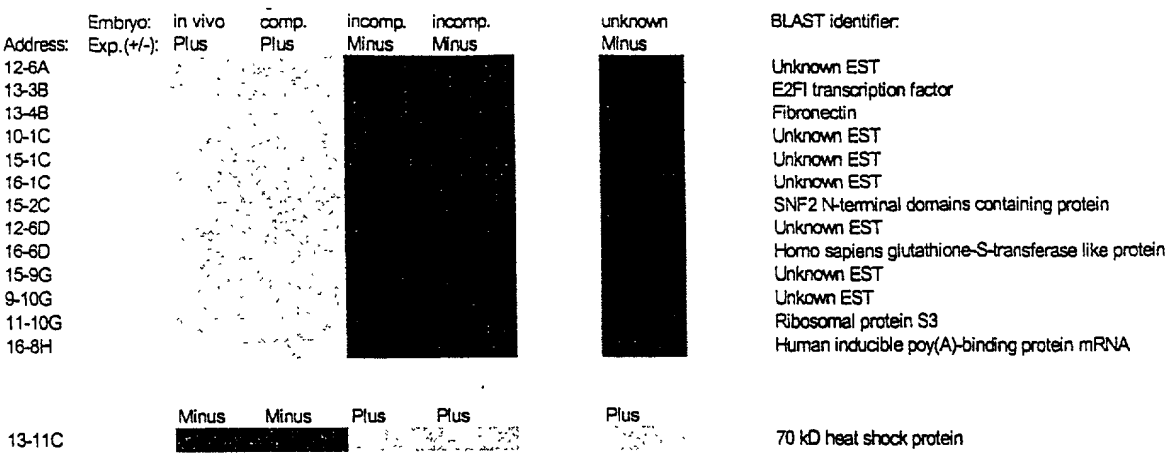


Figure 7: illustrates cluster analysis performed on individual embryos prepared by nuclear transfer using developmentally competent and incompetent cell lines, and embryos prepared by nuclear transfer using donor cells obtained from a cell of unknown developmental competence.